IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Gjalt W. Huisman, Laura Z. Luo and Oliver P. Peoples

Serial No.: 10/607,903 Art Unit: 1652

Filed: June 27, 2003 Examiner: Richard Hutson

For: *MICROBIAL STRAINS AND PROCESSES FOR THE MANUFACTURE OF BIOMATERIALS*

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. 1.132

Sir:

I, Wolfgang Liebl, hereby declare that:

1. I am a professor at the Institut für Mikrobiologie und Genetik, Georg-August-Universität, Grisebachstr. 8, D-37077 Göttingen, Germany.

2. I have worked for more than twenty years in the field of genetically engineered organisms for the production of polymers, including polyhydroxyalkanoates.

3. I do not have any financial interest in the above-referenced application or with Metabolix, Inc. the assignee.

4. I am the lead author of Liebl, *et al.*, *J. Bacteriology* 174(6):1854-1861 (1992) ("Liebl") (Abstract attached as Exhibit A). Liebl discloses Staphylococcal nuclease (SNase)

expression by various *C. glutamicum* strains, wherein the *C. glutamicum* transgenic strain is used for investigating protein export and processing. The nuclease in Liebl is secreted into the culture medium.

5. Figure 1 describes the construction of pWNuc5. It is my understanding that the examiner of the above-identified U.S. patent application cited Liebl, and in particular this figure and the data in Table 1, as "teaching expression of the heterologous nuclease (SNase) in *E. coli*.

6. This Figure describes the construction of inducible expression vectors for the expression of the SNase in *C. glutamicum*. pWNuc5 is an intermediate vector used in the last step of the vector construction process which is the insertion of the promoter and the lacIq repressor sequence to make the pWLQN2 plasmids used for the inducible expression studies subsequently reported for *C. glutamicum* transformed with these plasmids (Figs. 2 – 6).

7. When a promoter which is known to function in *E. coli* (Ptac) was inserted in front of the SNase gene for the first time, the lacIq repressor was simultaneously inserted which keeps the promoter inactive until it is induced by the addition of the inducing agent IPTG. No such induction studies are described for *E. coli* in the Liebl reference and therefore no expression of the SNase enzyme is described in the reference.

8. I declare that all statements made herein of my own knowledge and belief are true and that all statements made on information and belief are believed to be true, and further, that the statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 13 October 2009

Dr. Liebl Wolfgang

EXHIBIT A

J Bacteriol. 1992 March; 174(6): 1854-1861

research-article

Expression, secretion, and processing of staphylococcal nuclease by Corynebacterium glutamicum.

W Liebl, A J Sinskey and K H Schleifer

Lehrstuhl für Mikrobiologie, Technische Universität München, Germany.

ABSTRACT

The gene for staphylococcal nuclease (SNase), an extracellular enzyme of Staphylococcus aureus, was introduced into Corynebacterium glutamicum. The heterologous gene was expressed in this host organism, and SNase was efficiently exported to the culture medium. Amino-terminal sequencing of SNase secreted by C. glutamicum revealed that the signal peptide was apparently cleaved off at precisely the same position as in the original host, S. aureus. As with S. aureus, a second smaller form of SNase (A form), whose appearance is presumably the result of a secondary processing step, was found in the culture medium of the recombinant C. glutamicum strain. The A form was one residue shorter than the mature nuclease A produced by S. aureus. Variation of the sodium chloride concentration in the growth medium had a marked influence on the location and the processing of SNase by C. glutamicum. In a complex growth medium containing 4% sodium chloride, SNase was exclusively located in the supernatant, but a significant amount of the enzyme remained cell associated if the strain was grown in a low-salt medium. Also, high salt concentrations seemed to inhibit processing of the high-molecular-weight form of SNase (B form) to the smaller A form. Similarities and differences in the export and modes of processing of SNase by three different, nonrelated gram-positive host organisms are discussed. Finally, a versatile Escherichia coli-C. glutamicum tac-lacIq expression shuttle vector was constructed. With this vector, it was possible to achieve isopropyl-beta-D-galactopyranoside (IPTG)-inducible overexpression and secretion of SNase in C. glutamicum, whereby the expression level was dependent on the concentration of the inducer.

J Bacteriol. 1992 March; 174(6): 1854-1861